Free Rad. Res. Comms., Vol. 1, No. 4, pp. 273-287
8755-0199/86/0104-0273 $25.00/0

Printed in Great Britain

COMPARISON OF THE INACTIVATION OF MICROSOMAL GLUCOSE-6-PHOSPHATASE BY IN SITU LIPID PEROXIDATION-DERIVED 4-HYDROXYNONENAL AND EXOGENOUS 4-HYDROXYNONENAL

J.F. KOSTER[1,†], R.G. SLEE[1], A. MONTFOORT[2], J. LANG[3] and H. ESTERBAUER[3]

[1]*Department of Biochemistry I,* [2]*Department of Pathological Anatomy, Medical Faculty, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands, and* [3]*Institute für Biochemie, Universität Graz, Halbarthsgasse, A-8010 Graz, Austria.*

(Received October, 1st, 1985)

1) The effect of 4-hydroxynonenal and lipid peroxidation on the activities of glucose-6-phosphatase and palmitoyl CoA hydrolase were studied.

2) 4-Hydroxynonenal inactivates glucose-6-phosphatase but has no effect on palmitoyl-CoA hydrolase. These effects are similar with those observed during lipid peroxidation of microsomes.

3) The inhibition of glucose-6-phosphatase by 4-hydroxynonenal can be prevented by glutathione but not by vitamin E. The inactivation of glucose-6-phosphatase during lipid peroxidation is prevented by glutathione and delayed by vitamin E.

4) The formation of 4-hydroxynonenal during lipid peroxidation was followed in relation to the inactivation of glucose-6-phosphatase. At 50% inactivation of glucose-6-phosphatase the 4-hydroxynonenal concentration was 1.5 μM. To obtain 50% inactivation of glucose-6-phosphatase by added 4-hydroxynonenal a concentration of 150 μM or 300 μM was needed with a preincubation time of 30 and 60 min, respectively.

5) It is concluded that the glucose-6-phosphatase inactivation during lipid peroxidation can be due to the formation of 4-hydroxynonenal. The formed 4-hydroxynonenal which inactivates glucose-6-phosphatase is located in the membrane. If this mechanism is valid it implies that a functional SH group of glucose-6-phosphatase is layered in the membrane. However, an inactivation of glucose-6-phosphatase by desintegration of the membrane by lipid peroxidation cannot be ruled out.

Key words: lipid peroxidation, 4-hydroxynonenal, glucose-6-phosphatase, palmitoyl-CoA hydrolase.

INTRODUCTION

Lipid peroxidation of liver microsomes initiated by NADPH and Fe/ADP or certain haloalkanes is accompanied by the destruction of several membrane bound enzymes

† To whom correspondence should be addressed.

such as aminopyrine-demethylase, cytochrome P_{450} and glucose-6-phosphatase. Originally this impairment was thought to be due to destruction of the membrane phospholipids or by the free radicals directly[1]. However, during lipid peroxidation new substances are formed, which have cytopathological properties. The concept[2,3] that peroxidative decomposition of the phospholipids providing new substances with cytopathological properties has aroused great enthusiasm, since these substances can act as a type of metastable secondary messengers which can reach and affect targets far away, in molecular dimensions, from the site of their generation. So-called "long distance" pathological effects are observed *in vivo* after CCl_4 intoxication and *in vitro* with hepatocytes exposed to CCl_4, ADP/Fe^{2+} or other prooxidant stimuli. The most toxic component discovered so far in the complex mixture of lipid peroxidation-derived products is 4-hydroxynonenal (4-hydroxy2,3 transnonenal). This aldehyde is formed by peroxidizing microsomes[4] as well as by hepatocytes exposed to CCl_4 or ADP/Fe[5]. *In vitro* this aldehyde can mimic qualitatively many effects which are also observed under peroxidizing conditions. 4-Hydroxynonenal inhibits glucose-6-phosphatase[4], aminopyrine demethylase[4] and destructs cytochrome P_{450}[4], it has hemolytic activity[4] and inhibits protein, DNA and RNA synthesis[6]. In addition many other biological effects exhibited by hydroxynonenal are reported[7].

With few exceptions (inhibition of adenylate cyclase, chemotoxic activity) hydroxynonenal shows effects on isolated enzymes, subcellular fractions and cells only at concentration in the range of 100 μM or above. It was questioned[8] therefore if such a relatively high needed concentration can be generated in the lipid peroxidation process in particular if lipid peroxidation is minimal and restricted to small areas as it is the case with CCl_4 or $BrCCl_3$ metabolism.

The goal of this paper is to investigate how far 4-hydroxynonenal can be responsible for the described effect. The approach we took was to compare the generation of 4-hydroxynonenal in microsomes related to the inhibition of glucose-6-phosphatase and the concentration needed of added 4-hydroxynonenal to obtain the same extent of inhibition.

MATERIALS AND METHODS

Rat (Wistar, 180–220 g) liver microsomes were isolated from liver homogenate (in 0.25 M surcrose) by differential centrifugation. The microsomes were washed and finally taken up in 0.15 M KCl.

Lipid peroxidation was performed in 30 mM Tris/HCl (pH 7.4) in the presence of 0.374 mM Fe^{3+}/ADP (10 mM) with either NADPH[9] or a NADPH regenerating system[10]. Experimental samples and controls were incubated at 37°C under shaking and free excess of air. At the indicated times samples were taken to measure the content of 4-hydroxynonenal, malondialdehyde and the activities of glucose-6-phosphatase and palmitoyl-CoA hydrolase.

4-Hydroxynonenal content was determined by HPLC[11] and malondialdehyde was measured as described elsewhere[9]. To calculate the amount of malondialdehyde formed the millimolar extinction coefficient of 156 is used.

Glucose-6-phosphatase activity was measured according to Harper[12] and palmitoyl-CoA hydrolase according to Jansen & Hülsmann[13].

Incubations of microsomes (1 mg protein/ml) with 4-hydroxynonenal were carried out at 37°C under shaking in 50 mM Tris-HCl (pH 6.6), 0.15 M KCl and 6 mM

EDTA. Samples were taken at the indicated times to measure the activities of glucose-6-phosphatase and palmitoyl-CoA hydrolase and 4-hydroxynonenal.

RESULTS

1) Kinetics of the Inactivation of Glucose-6-Phosphatase in Relation to the Formation of Malondialdehyde and 4-Hydroxynonenal during Microsomal Lipid Peroxidation

Figure 1 shows that liver microsomal glucose-6-phosphatase is rapidly inactivated during lipid peroxidation by NADPH and ADP/Fe as a prooxidant stimulus. The

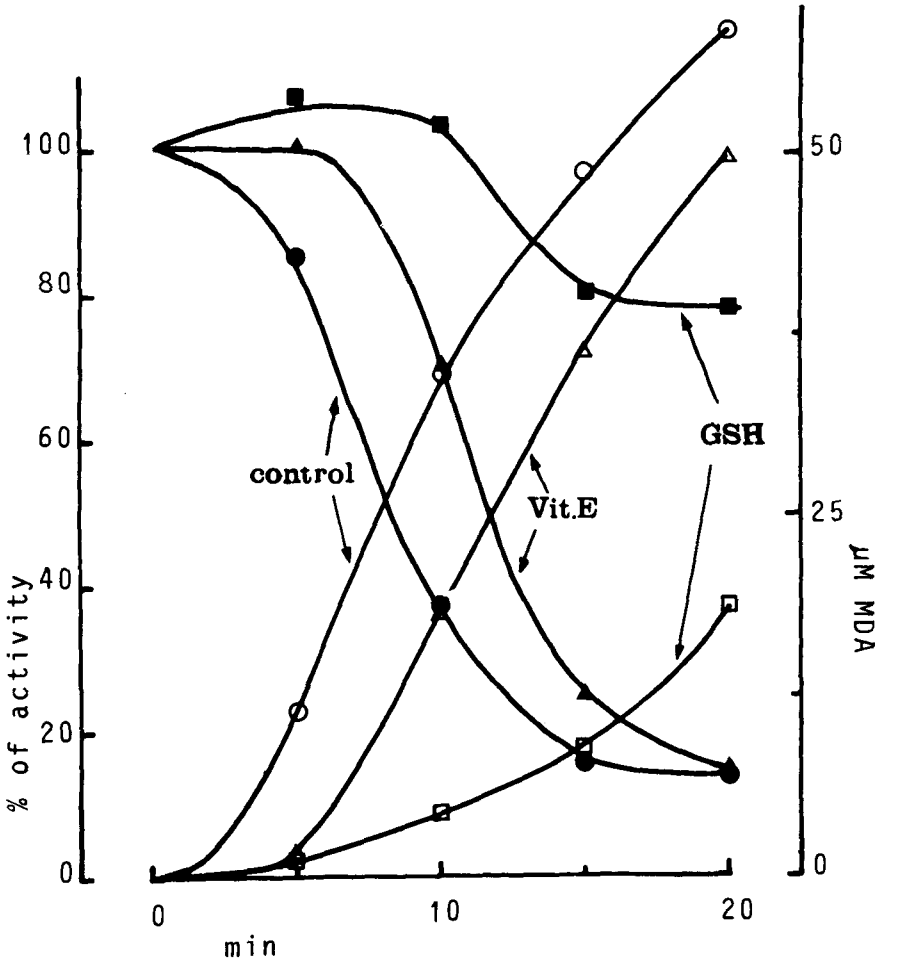

FIGURE 1 The influence of vitamin E (1 mM) and reduced glutathione (5 mM) on the generation of malondialydehyde and the inactivation of glucose-6-phosphatase during lipid peroxidation. ○-○, control; △-△, + vitamin E and □-□, + reduced gluthathione. Open symbols indicate the malondialdehyde formation and closed symbols the glucose-6-phosphatase activity.

lipid peroxidation has been followed by the production of malondialdehyde. The inhibition, which has been reported by several other authors[14,9] appears to be related with the lipid peroxidation process. In incubation systems, supplemented with vitamin E, a well known free radical scavenger, the onset of lipid peroxidation is delayed for about 5 min and this is also reflected on the onset of the inactivation of glucose-6-phosphatase. In both systems (with and without vitamin E) the loss of glucose-6-phosphatase activity was equal after 20 min while the extent of lipid peroxidation in the presence of vitamin E was slightly lower (13%) as indicated by the malondialdehyde produced. Glutathione effectively prevents the lipid peroxidation and concomitant the inactivation of glucose-6-phosphatase. After 20 min incubation the loss of glucose-6-phosphatase activity and the malondialdehyde formation was only 20% and 30% respectively of the control values.

It is known[15] that lipid peroxidation produces a wide range of more or less toxic lipid degradation products. Elegant studies by Benedetti *et al.*[2,3] showed that

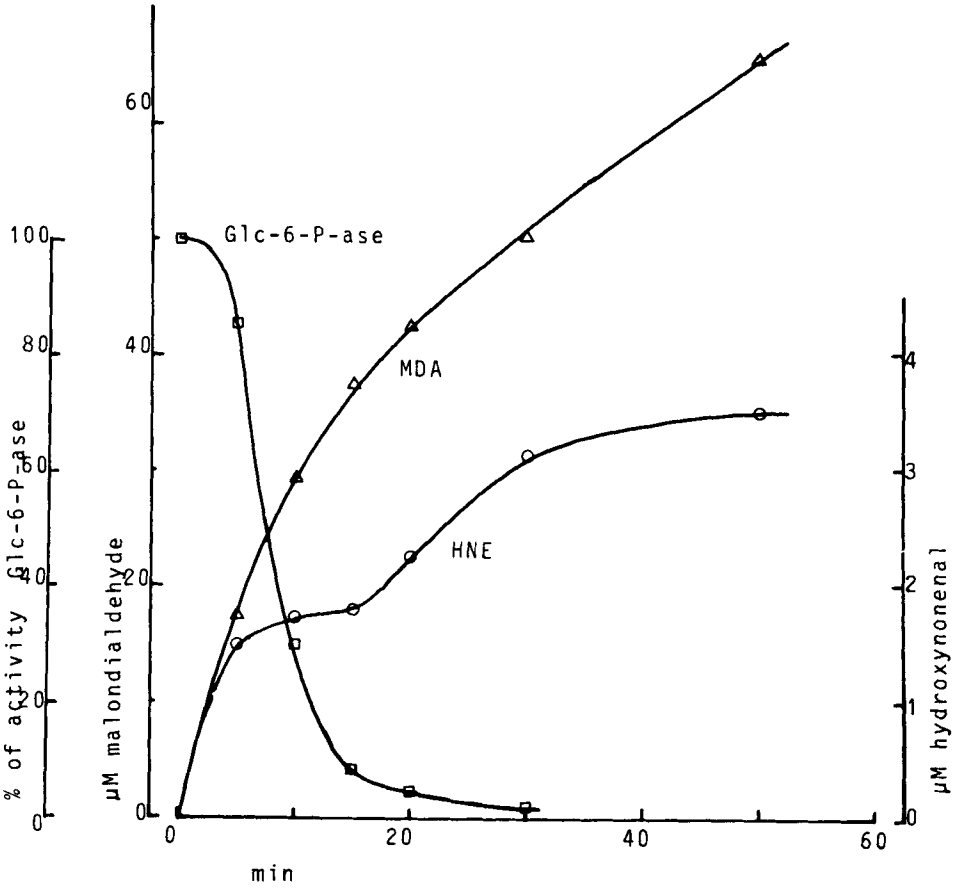

FIGURE 2 The formation of malondialdehyde and 4-hydroxynonenal during peroxidation of microsomes. For conditions see Materials and Methods section. Note the difference in scale for malondialdehyde and malondialdehyde △-△, malondialdehyde; ○-○, 4-hydroxynonenal and □-□, inactivation of glucose-6-phosphatase as % of the original activity.

peroxidizing microsomes release water soluble substances in the medium, which have the capacity to inhibit glucose-6-phosphatase. The substance with the highest inhibitory activity was isolated and identified as 4-hydroxynonenal[4,16]. The biogenic aldehyde as well as 4-hydroxynonenal prepared by chemical synthesis are equally effective[4]. However, the dose needed to gain 50% inhibition of glucose-6-phosphatase activity is rather high (> 100 μM) and a preincubation of 60 min is needed. It was therefore questioned if during lipid peroxidation and high concentrations of 4-hydroxynonenal are reached to eliminate glucose-6-phosphatase. With a newly developed method[11] we were able to measure quantitatively the concentrations of 4-hydroxynonenal in microsomes exposed to various prooxidant stimuli. Figure 2 exhibits the malondialdehyde and 4-hydroxynonenal formation during microsomal lipid peroxidation induced by NADPH and ADP/Fe^{3+}. It shows that the amount of 4-hydroxynonenal formed is about 10% of the malondialdehyde formation and reaches a value in the range of 1.5–3.5 μM. It should be noted that the assay measures the 4-hydroxynonenal dissolved in the aqueous phase of the incubation medium together with the amount enclosed within the microsomal membrane. It should be emphasized that 4-hydroxynonenal can be unequally distributed over the two phases and through its lysophilic nature more or less accumulated in the lipid domains of the microsomal membrane[16,17].

In order to compare the various data we have normalized all data by putting all values reached at 20 min at 100%. At 20 min practically all glucose-6-phosphatase activity (98%) is lost and all later events are not significant for the mechanism by which the enzyme is inactivated. It should also be noted that most of the polyunsaturated fatty acids are peroxidatively decomposed in the first 20 to 30 min of incubation[16,18]. The kinetic analysis depicted in Figure 3 clearly shows a close time link between the three parameters. The shapes of the curves are not significantly changed by setting the 30 min values at 100%. The 4-hydroxynonenal is apparently a very early event, half of the 4-hydroxynonenal is already generated in the first 4 min while the inhibition of glucose-6-phosphatase is only 10%. This fast production seems to support the hypothesis that the inactivation of glucose-6-phosphatase is causally linked with the generation of the aldehyde. Assuming that the aldehyde inactivates glucose-6-phosphatase by blocking its functional SH groups[19] the onset of inhibition must be delayed since the binding to the SH-group will need some time. It is of interest that the formation of 4-hydroxynonenal is biphasic. After a rapid formation within the first 5 min the production ceases more or less for the next 10 min and then continues again. It is also remarkable that before the second phase starts, the inactivation of glucose-6-phosphatase is complete. It seems likely that the two phase kinetics reflect two distinct stages. The initial rapid evolution of 4-hydroxynonenal emerges from minimal lipid peroxidation restricted to specific and distinct site of the membrane. This is substantiated by the fact that the end of the first phase glucose-6-phosphatase is completely inhibited. This specific process yields about 1.7 nmol 4-hydroxynonenal per mg microsomal protein. After this phase lipid peroxidation spreads out along the entire protein-lipid membrane leading to unspecific peroxidation of polyunsaturated fatty acids, which decompose in part again to 4-hydroxynonenal. This latter process continues slowly up to at least 60 min.

The biphasic formation of 4-hydroxynonenal is not restricted to lipid peroxidation induced by NADPH and ADP/Fe^{3+}. Also microsomes exposed to CCl_4 and $BrCCl_3$ as prooxidative stimuli exhibit the characteristic biphasic behaviour (Esterbauer *et al.*, unpublished results).

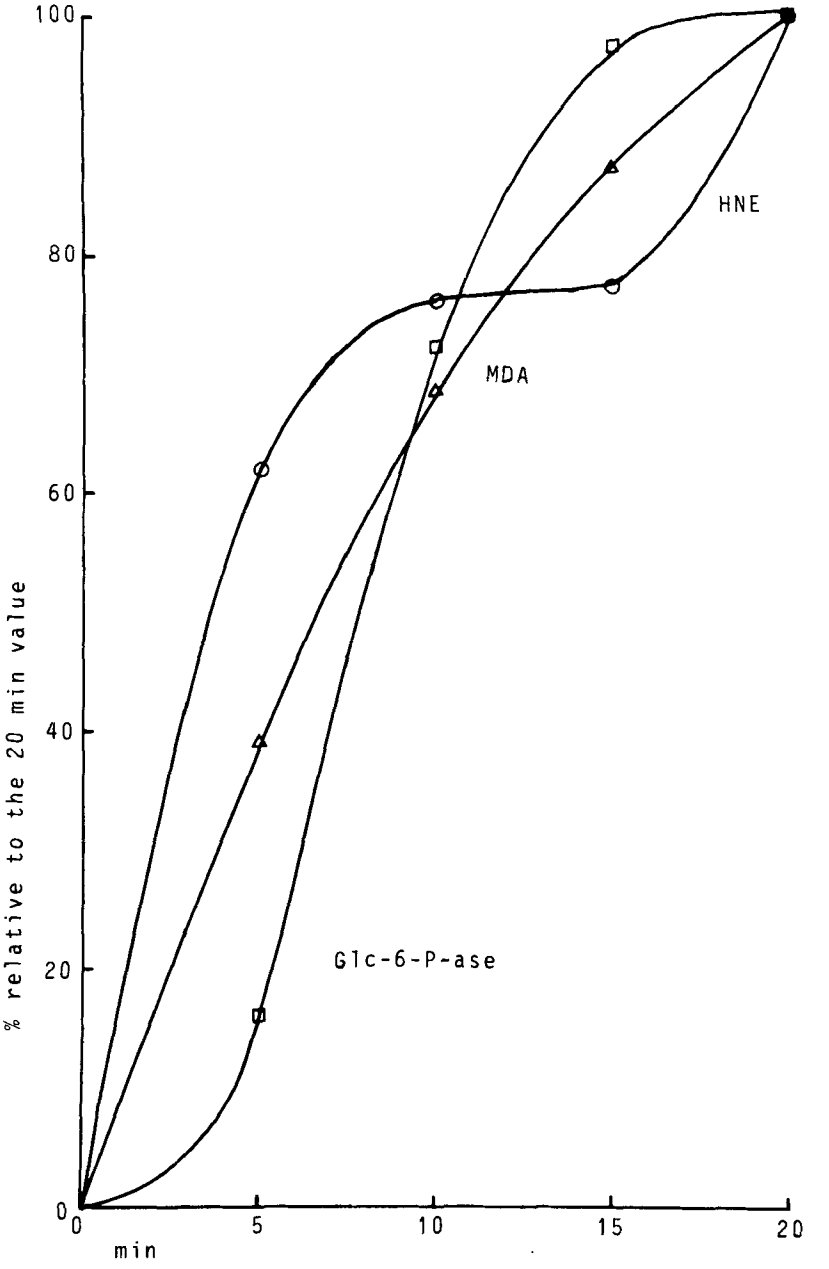

FIGURE 3 Kinetics of the formation of malondialdehyde, 4-hydroxynonenal and the inactivation of glucose-6-phosphatase during microsomal lipid peroxidation. The percentages are calculated by putting the 20 min value for 100%. △-△, malondialdehyde; ○-○, 4-hydroxynonenal and □-□, glucose-6-phosphatase.

2) *The Effect of Synthetic 4-Hydroxynonenal on Microsomal Glucose-6-Phosphatase and Palmitoyl-CoA Hydrolase*

Liver microsomes are incubated with various amounts of synthetic 4-hydroxynonenal (0.1 to 1 mM) for 60 min, after which the activities of glucose-6-phosphatase and palmitoyl-CoA hydrolase are measured (Figure 4). In agreement with Benedetti *et al.*[4] 4-hydroxynonenal inactivates glucose-6-phosphatase in a dose-dependent manner. Under these conditions the concentration needed for 50% inactivation is about 175 μM. Even at the highest concentrations of 4-hydroxynonenal still about 18% of the glucose-6-phosphatase activity has remained. Under conditions of lipid peroxidation the remaining activity of glucose-6-phosphatase is about 10%. Palmitoyl-CoA hydrolase, another microsomal enzyme, is not significantly attacked by 4-hydroxynonenal. At the highest concentration of 4-hydroxynonenal (1 mM) an inhibition of 10% is observed. These findings indicate that 4-hydroxynonenal is not merely a toxic unspecific enzyme blocker as for example other aldehydes which cross-link and thereby inactivates enzymes [see Ref. 7]. In experiments where lipid peroxidation was

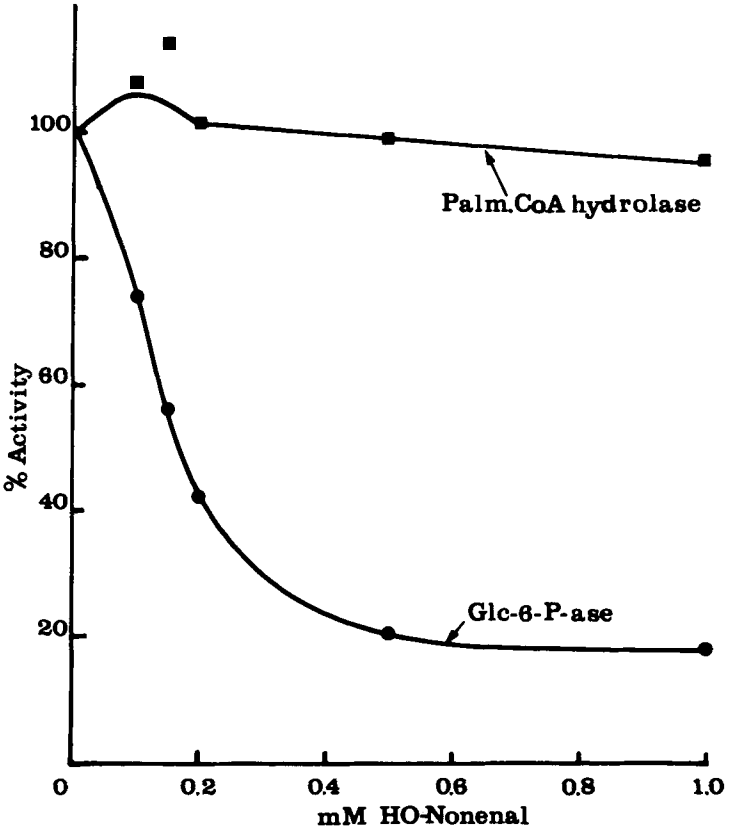

FIGURE 4 The effect of 4-hydroxynonenal and the activities of glucose-6-phosphatase and palmitoyl-CoA hydrolase. The microsomes have been preincubated with 4-hydroxynonenal for 60 min at 37°C. ●-●, glucose-6-phosphatase and ■-■, palmitoyl-CoA hydrolase.

induced by NADPH and ADP/Fe^{3+} palmitoyl-CoA hydrolase activity remains rather stable[9].

The effect of 4-hydroxynonenal on the two microsomal enzymes, i.e. glucose-6-phosphatase and palmitoyl-CoA hydrolase, mimics, at least qualitatively, the effect of lipid peroxidation on these two enzymes.

The mechanism by which 4-hydroxynonenal inhibits glucose-6-phosphatase is not clear. The aldehyde is an electrophylic agent and shows high reactivity towards SH group in proteins and low-molecular weight thiols, such as cysteine and glutathione[20,21]. It was therefore suggested that the enzyme is inactivated by a blockade of a functional SH group, as shown by Cori *et al.*[19]. Treatment of microsomes with the SH blocker p-chloromercuric benzoate leads also to an inactivation, which cannot be reversed by cysteine or dithiothreitol. Cadenas *et al.*[22] show that 4-hydroxynonenal enhances chemiluminiscence and pentane production in isolated hepatocytes. Since both parameters are generally recognized as an index for lipid peroxidation processes it can be possible that 4-hydroxynonenal acts as an initiator of lipid peroxidation in the hepatocytes. We, therefore, tested the effects of 4-hydroxynonenal on microsomal glucose-6-phosphatase in the presence and absence of externally added vitamin E. As can be seen in Figure 5, vitamin E (4 mM) cannot prevent the loss of glucose-6-phosphatase activity caused by 1 mM 4-hydroxynonenal. It can therefore be ruled out with some certainty that the inactivation of glucose-6-phosphatase by synthetic 4-hydroxynonenal is brought about by radical-induced lipid peroxidation processes. Glutathione (5 mM) completely abolishes the inhibitory action of 4-hydroxynonenal. This was expected since glutathione rapidly reacts with the aldehyde to give a Michael adduct[20].

Interaction of 4-hydroxynonenal with microsomes That 4-hydroxynonenal in fact interact with microsomal constituents is shown in Figure 5. The consumption of 4-hydroxynonenal is followed in an incubation system containing microsomes and respectively 10 and 50 μM 4-hydroxynonenal. The time curve shows that the interaction of 4-hydroxynonenal and microsomes is a rather slow process. With 10 μM the consumption of 4-hydroxynonenal still continues even after 60 min of incubation, whereas with 50 μM, the consumption is more or less complete after 60 mins. Since the assay used for 4-hydroxynonenal analyses measures total free 4-hydroxynonenal, this consumption caused by microsomes can only be due to a binding with proteins and/or phospholipids. A metabolism by the mixed function oxidase or aldehyde dehydrogenases is very unlikely since the system did not contain electron donating cofactors, but a possible action of aldehyde oxidases cannot be ruled out.

It has been shown previously by Benedetti *et al.*[23] that microsomes peroxidized by ADP/Fe for 20 mins contain 38 nmol protein bound aldehydes per mg protein[23] and 315 nmol carbonyl functions per mg phospholipid[24]. Compared to this value the binding of externally added 4-hydroxynonenal is rather low. For the 60 min values of the curves shown in Figure 5 it can be deduced that only 6 (incubation with 10 μM) and 15 nmol (incubation with 50 μM) of 4-hydroxynonenal are consumed per mg microsomal protein, most likely by chemical binding to proteins. One must therefore assume that in peroxidizing microsomes in addition 4-hydroxynonenal many other aldehydes capable to attack membrane proteins are generated. In view of this the inactivation of glucose-6-phosphatase by lipid peroxidation can be a cumulative or synergisitc aldehyde effect, wherein 4-hydroxynonenal play an important but yet not the only role.

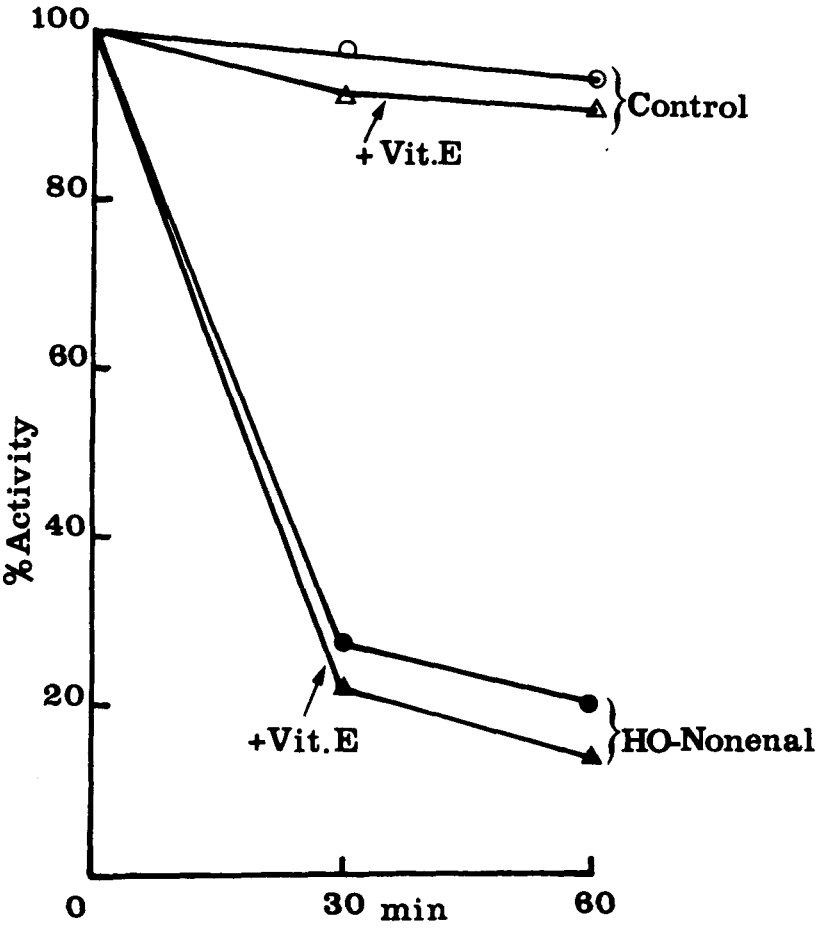

FIGURE 5 The influence of vitamin E (4 mM) on the inactivation of glucose-6-phosphates by preincubation with 4-hydroxynonenal (1·mM), ○-○, control; △-△, control + vitamin E, ●-●, with 4-hydroxynonenal and ▲-▲, with 4-hydroxynonenal + vitamin E.

4-Hydroxynonenal possesses an asymmetric centre at carbon 4 and therefore exists in two stereoisomers. The synthetic 4-hydroxylnonenal is not known, but the possibility exists that lipid peroxidation produces only one stereoisomers, which theoretically can be a more potent inhibitor of glucose-6-phosphatase than its stereo opponent. However, the possible difference with the racemate cannot be more than twofold.

3) *Comparison of Inactivation of Glucose-6-Phosphatase by Lipid Peroxidation and by Added 4-Hydroxynonenal*

As shown above, lipid peroxidation leads to a rapid inactivation of microsomal glucose-6-phosphatase and this inactivation is timely linked with the generation of

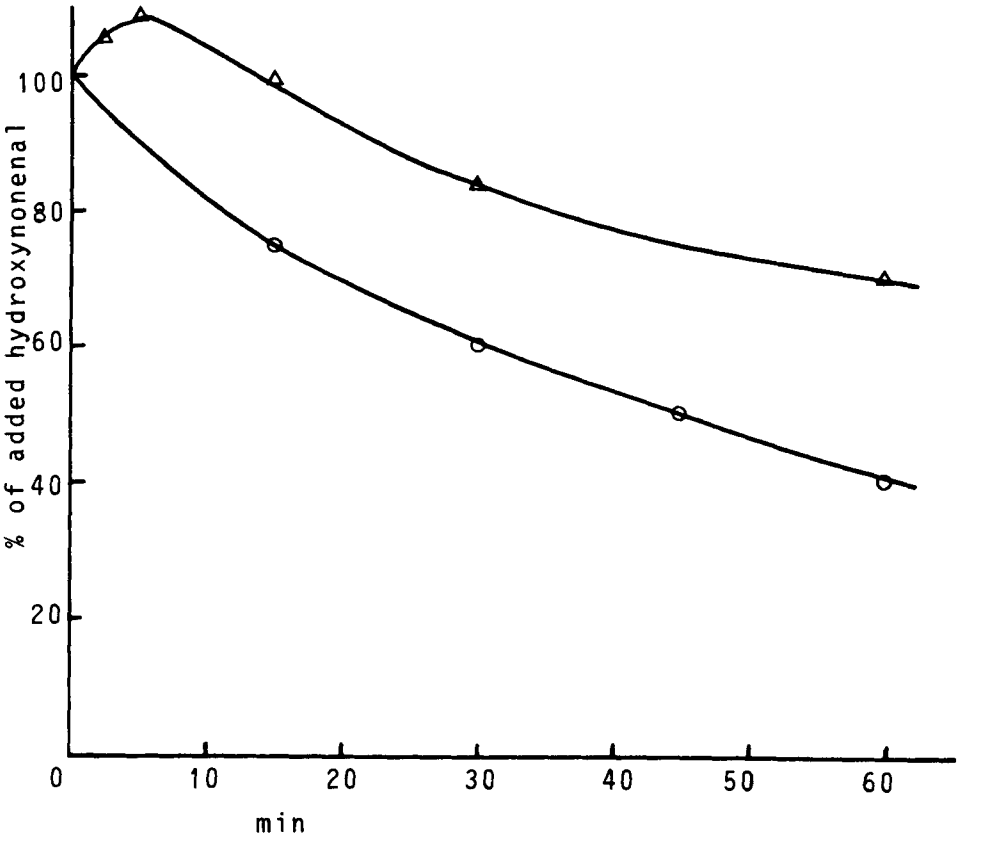

FIGURE 6 The disappearance of 4-hydroxynonenal during incubation with microsomes. The values are given as percentages of the added amount of aldehyde. ○-○, 10 μM and △-△, 50 μM 4-hydroxynonenal. Microsomes were in concentrations of 1 mg/ml.

the toxic aldehyde 4-hydroxynonenal. Plotting the amount of 4-hydroxynonenal generated during lipid peroxidation against the corresponding loss of glucose-6-phosphatase activity a "dose effect" curve is obtained [Figure 7]. Presumed that the 4-hydroxynonenal is the principal agent inhibiting glucose-6-phosphatase the ID_{50} must be about 1.5 μM. On the other hand if freshly prepared microsomes are incubated under non-peroxidizing conditions in systems supplemented with 4-hydroxynonenal the concentrations needed to obtain substantial inactivation of glucose-6-phosphatase are about two order of magnitude higher. The ID_{50} depends on the preincubation time and was 300 μM with 30 min and 175 μM with 60 min of preincubation. As expected from the rather low rate of binding of the aldehyde the microsomal constituents [Figure 5], the inactivation of glucose-6-phosphatase is not a very fast process. As depicted in Figure 8 with doses of 100, 150 and 200 μM 4-hydroxynonenal the inhibition increases linearly with time at least up to 60 min. Noteworth is also the different stage of the dose effect curves for generated 4-hydroxynonenal and added 4-hydroxynonenal. The curve for 4-hydroxynonenal

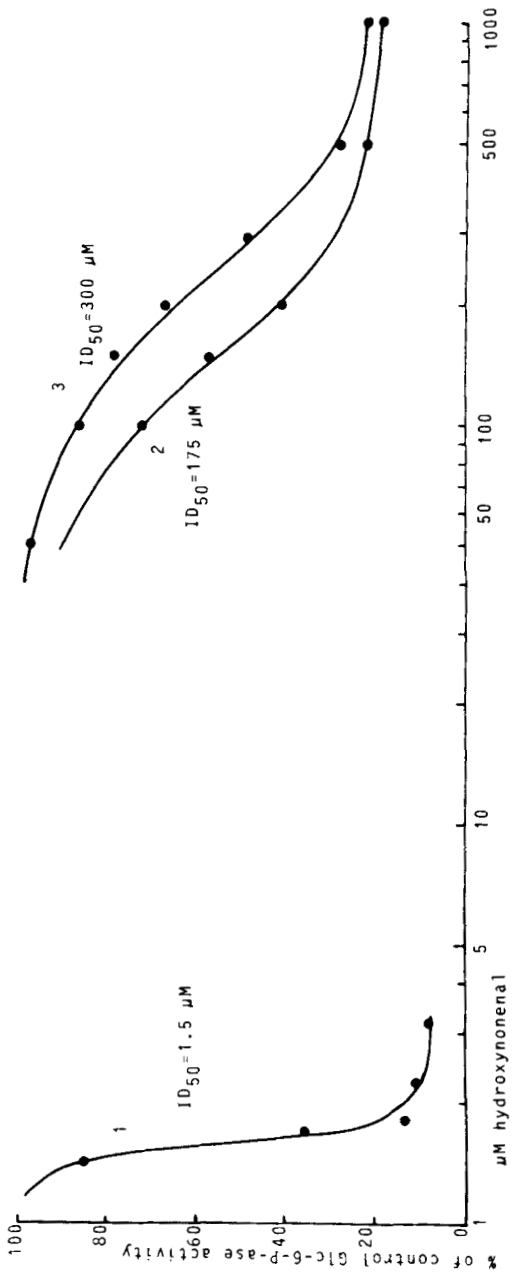

FIGURE 7 Relation between the concentration of 4-hydroxynonenal and the inactivation of glucose-6-phosphatase. Curve 1: 4-hydroxynonenal concentration as generated by microsomes during lipid peroxidation. Curve 2: microsomes incubated for 60 min with added 4-hydroxynonenal and curve 3: microsomes incubated for 30 min with added 4-hydroxynonenal.

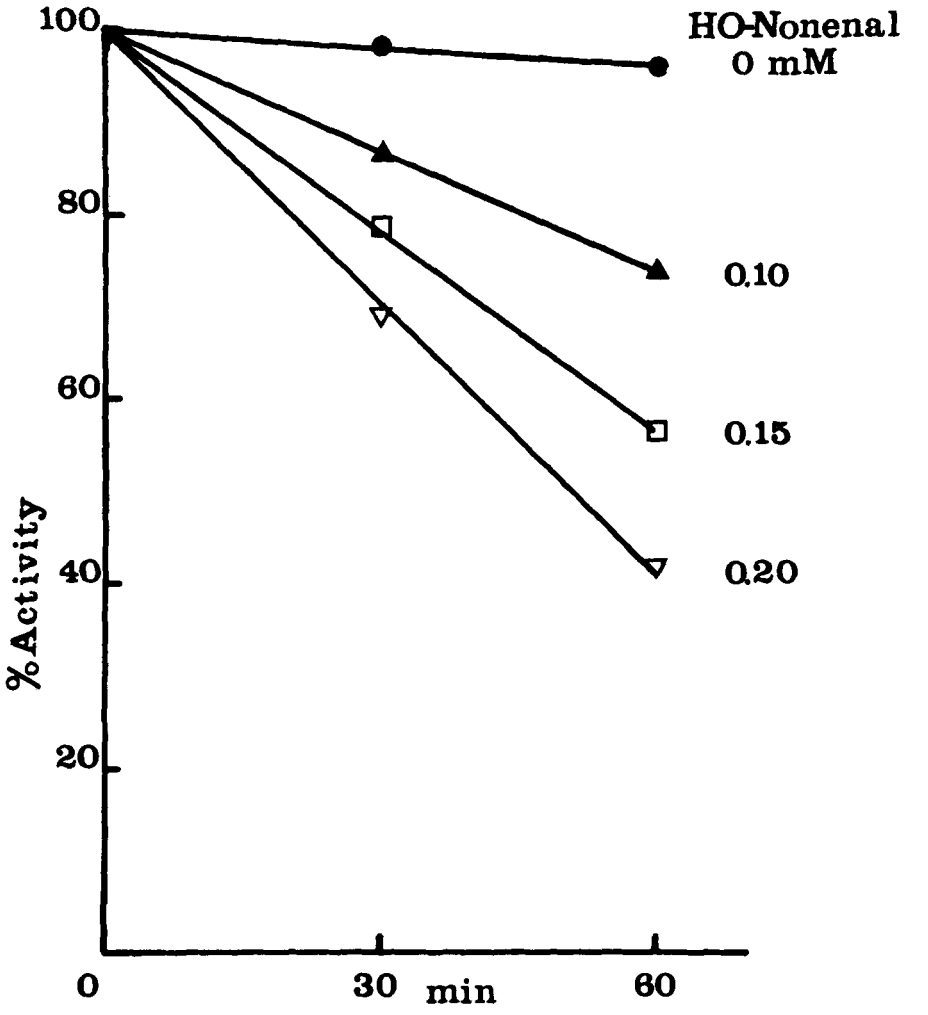

FIGURE 8 The incubation of glucose-6-phosphatase versus the preincubation time with various concentrations of 4-hydroxynonenal. ●-●, 0 mM; ▲-▲, 0.10 mM; □-□, 0.15 mM and ▽-▽, 0.2 mM.

generated by lipid peroxidation lies in a narrow range of concentrations (1–2 μM), whereas the curve for the added 4-hydroxynonenal is rather flat and covers a wide concentration range from 50 to 500 μM.

This comparison between in situ generated 4-hyroxynonenal and externally added 4-hydroxynonenal clearly shows remarkable differences in the effective dose, the kinetics of the establishment of the inactivation and the form of the dose inactivation profile.

DISCUSSION

The main goal of the present study is to investigate whether 4-hydroxynonenal can be held responsible for the inactivation of glucose-6-phosphatase, occurring during lipid peroxidation. The recently developed procedure for measuring low amounts of 4-hydroxynonenal in biological samples enabled us to follow quantitively the kinetics of 4-hydroxynonenal formation during lipid peroxidation. The amount of 4-hydroxynonenal generated in the microsomes during NADPH, ADP/Fe induced lipid peroxidation is about 10% of the concomitantly generated malondialdehyde. Presuming that 4-hydroxynonenal is the principal agent, which destroys glucose-6-phosphatase during lipid peroxidation, means that to obtain 50% inhibition 1.5 μM of 4-hydroxynonenal is needed. This is in sharp contrast to the 175 μM (60 min preincubation) and 300 μM (30 min preincubation) of 4-hydroxynonenal needed when this agent is added to microsomes to reach 50% inactivation of glucose-6-phosphatase. From this quantitive comparison of dose effect any mechanism for explaining lipid peroxidation-dependent loss of glucose-6-phosphatase must be ruled out, which implies that 1) 4-hydroxynonenal is generated in the membrane; 2) released into the aqueous medium and 3) targets glucose-6-phosphatase from the aqueous phase (see Figure 9).

The inactivation of glucose-6-phosphatase is complete in about 10 min after the onset of lipid peroxidation and 4-hydroxynonenal formation, whereas externally added 4-hydroxynonenal (concentrations below 200 μM) inactivates the enzyme only slowly and gradually. This latter is in agreement with the finding that binding of 4-hydroxynonenal to microsomal constituents is also found to be a rather slow process. In view of this it seems also unrealistic that 4-hydroxynonenal generated in the endoplasmatic reticulum of hepatocytes or intact liver can build up in the cytoplasm concentrations sufficient for enzyme inactivation. Moreover, it was

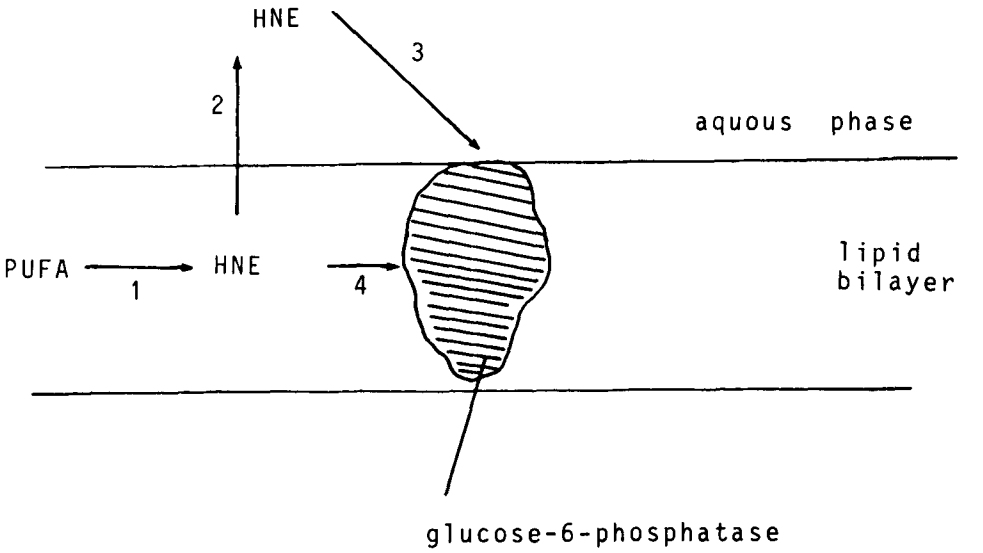

FIGURE 9 A schematic presentation for the inactivation pathway of microsomal glucose-6-phosphatase by 4-hydroxynonenal. Pathways 1, 2, and 3 are ruled out, but pathways 1 and 4 are quite possible.

found[25] that liver cells possess a high capacity to detoxify the aldehyde within the endoplasmatic reticulum by a reductive pathway.

Comparing the effects of *in situ* generated 4-hydroxynonenal with the effect of exogenous 4-hydroxynonenal is hampered for several reasons. The two systems are not fully comparable. Through its lipophilic nature most of the 4-hydroxynonenal generated during lipid peroxidation actually remains in the lipid core of the microsomes[16], where its local concentration can be, at least for a limited time, orders of magnitude above that in the aqueous phase. Benedetti *et al.*[36] have estimated that in situations where peroxidation is minimal (CCl_4, $BrCCl_3$, 6 μM Fe, 20 min incubations) the amount of 4-hydroxynonenal is about 100 times higher in the microsomal pellet than in the aqueous phase. In our experiments microsomes generated 1.5 nmol 4-hydroxynonenal per mg protein within the first 5 min. With the assumption that no significant quantities diffused into the surrounding medium and a protein lipid ratio of 3:1[27] the concentration of 4-hydroxynonenal equally distributed along lipid bilayer will be 4.5 mM. It is more or less accepted that the early stage of lipid peroxidation is restricted to limited membrane sites. The local concentration of 4-hydroxynonenal near or at the site of membrane-embedded parts of enzymes can therefore be even much higher than 4.5 mM. It is clear that such a peculiar situation within the membrane cannot be mimicked in experiments with microsomes and externally added 4-hydroxynonenal. Needless to say that also the dynamics of the two systems will be quite different.

Based on our experimental results and considerations we can rule out with certainty the inactivation mechanism for glucose-6-phosphatase by 4-hydroxynonenal which involves: formation of 4-hydroxynonenal in the microsomal membranes by lipid peroxidation, release of aldehyde into the cytosol and attack the enzyme at the region exposed to the aqueous phase. This is visualized in Figure 9 at pathways 1, 2 and 3. The second mechanism (pathways 1 and 4, Figure 9), which assumes that the locally produced 4-hydroxynonenal attacks hydrophobic region of the enzyme is quite possible. This probably also indicates that a functional SH group is positioned in the membrane. However, with the present knowledge pertaining the site of inhibition of the enzyme, role of diffusion of 4-hydroxynonenal over the membrane, its diffusion out of the membrane and its distribution constant between the phospholipid and the aqueous it must remain open whether this mechanism is valid. Finally it is possible that a completely other mechanism not directly and casually associated with 4-hydroxynonenal can be responsible for the inactivation of glucose-6-phosphatase and other cytopathological effects associated with lipid peroxidation. A still possible mechanism can be the membrane distortion occurring due to the formation of peroxidized fatty acids. For these latter it is suggested[28] that they form clusters in the membrane, through which Ca^{2+} can be transported, which can result in an activation of phospholipase A_2.

Acknowledgements

Part of this work was supported by a research contract from the National Foundation for Cancer Research, Bethesda, USA.

References

1. B.A. Freeman and J.D. Crapo, *Lab. Invest.*, **47**, 412–426, (1982).
2. A. Benedetti, A.F. Casini and M. Ferrati, *Res. Commun. Chem. Pathol. Pharmacol.*, **17**, 519–528, (1977).
3. A. Benedetti, A.F. Casini, M. Ferrati and M. Comporti, *Biochem. J.*, **180**, 303–312, (1979).
4. A. Benedetti, M. Comporti and H. Esterbauer, *Biochim. Biophys. Acta*, **620**, 281–296, (1980).
5. G. Poli, M.U. Dianzani, K.H. Cheesman, T.F. Slater, J. Lang and H. Esterbauer, *Biochem. J.*, in press.
6. E. Schauerstein and H. Esterbauer, in *Subcellular Biology and Cancer*, Ciba Foundation Series, vol. 67, Excerpta Medica, Elsevier (Amsterdam), pp. 225–244, (1974).
7. E. Schauenstein, H. Esterbauer and H. Zollner, *"Aldehydes in Biological Systems"*, Pion Limited, London, (1977).
8. R.O. Recknagel, *Trends Pharmacol. Sci.*, **4**, 129–131, (1983).
9. J.F. Koster and R.G. Slee, *Biochim. Biophys. Acta*, **620**, 489–499, (1980).
10. T.F. Slater and Sawyer, *Biohem. J.*, **123**, 805–814, (1971).
11. J. Lang and H. Esterbauer, submitted for publication.
12. A.E. Harper, in *Methoden der enzymatischen Analyse*. Ed. H.U. Bergmeyer, 2nd edn., pp. 788–792, Verlag Chemie, Weinheim, (1965).
13. H. Jansen and W.C. Hülsmann, *Biochim. Biophys. Acta*, **296**, 241–248, (1973).
14. E.D. Wills, *Biochem. J.*, **123**, 983–991, (1971).
15. H. Esterbauer, in *Free Radicals, Lipid Peroxidation and Cancer* (D.C.H. McBrian, and T.F. Slater, eds.) 129–158, (1982).
16. H. Esterbauer, K.H. Cheesman, M.U. Dianzani, G. Poli and T.F. Slater, *Biochem. J.*, **208**, 129–140, (1982).
17. A. Benedetti, M. Comporti, R. Fulcari and H. Esterbauer, *Biochim. Biophys. Acta*, **792**, 172–181, (1984).
18. D.J. Kornburst and R.D. Mavis, *Mol. Pharmacol.*, **17**, 408–419, (1980).
19. C.F. Cori, R.C. Garland and H.W. Chang, *Biochem.*, **12**, 3126–3130, (1977).
20. H. Esterbauer, H. Zollner and N. Scholtz, *Z. Naturforschung*, **30c**, 466–473, (1975).
21. H. Esterbauer, A. Ertl and N. Schotz, *Tetrahedron*, **32**, 285–289, (1976).
22. E. Cadenas, A. Müller, R. Brigelius, H. Esterbauer and H. Sies, *Biochem. J.*, **214**, 479–487, (1983).
23. A. Benedetti, H. Esterbauer, M. Ferrati, R. Fulceri and M. Comporti, *Biochim. Biophys. Acta*, **711**, 345–356, (1982).
24. A. Benedetti, R. Fulceri, M. Ferrati, C. Ciccoli, H. Esterbauer and M. Comporti, *Biochim. Biophys. Acta*, **712**, 628–638, (1982).
25. J. Lang, H. Zollner and H. Esterbauer, *Biochem. J.*, in press.
26. A. Benedetti, R. Fulceri, M. Ferrati and M. Comporti, *Chem. -Biol. Interaction*, in press.
27. J.W. Depierre and G. Dallner, *Biochim. Biophys. Acta*, **415**, 411–472, (1975).
28. F.Z. Meerson, V.E. Kagan, Y.P. Kazlov, L.M. Belkina and Y.V. Arkhipenko, *Basic Res. Cardiol.*, **77**, 465–485, (1982).

Accepted by Dr. J.V. Bannister